Current Biology, Volume *28*

Supplemental Information

The Microtubule-Associated Protein CLASP Sustains Cell Proliferation through a Brassinosteroid Signaling Negative Feedback Loop

Yuan Ruan, Laryssa S. Halat, Deirdre Khan, Sylwia Jancowski, Chris Ambrose, Mark F. Belmonte, and Geoffrey O. Wasteneys

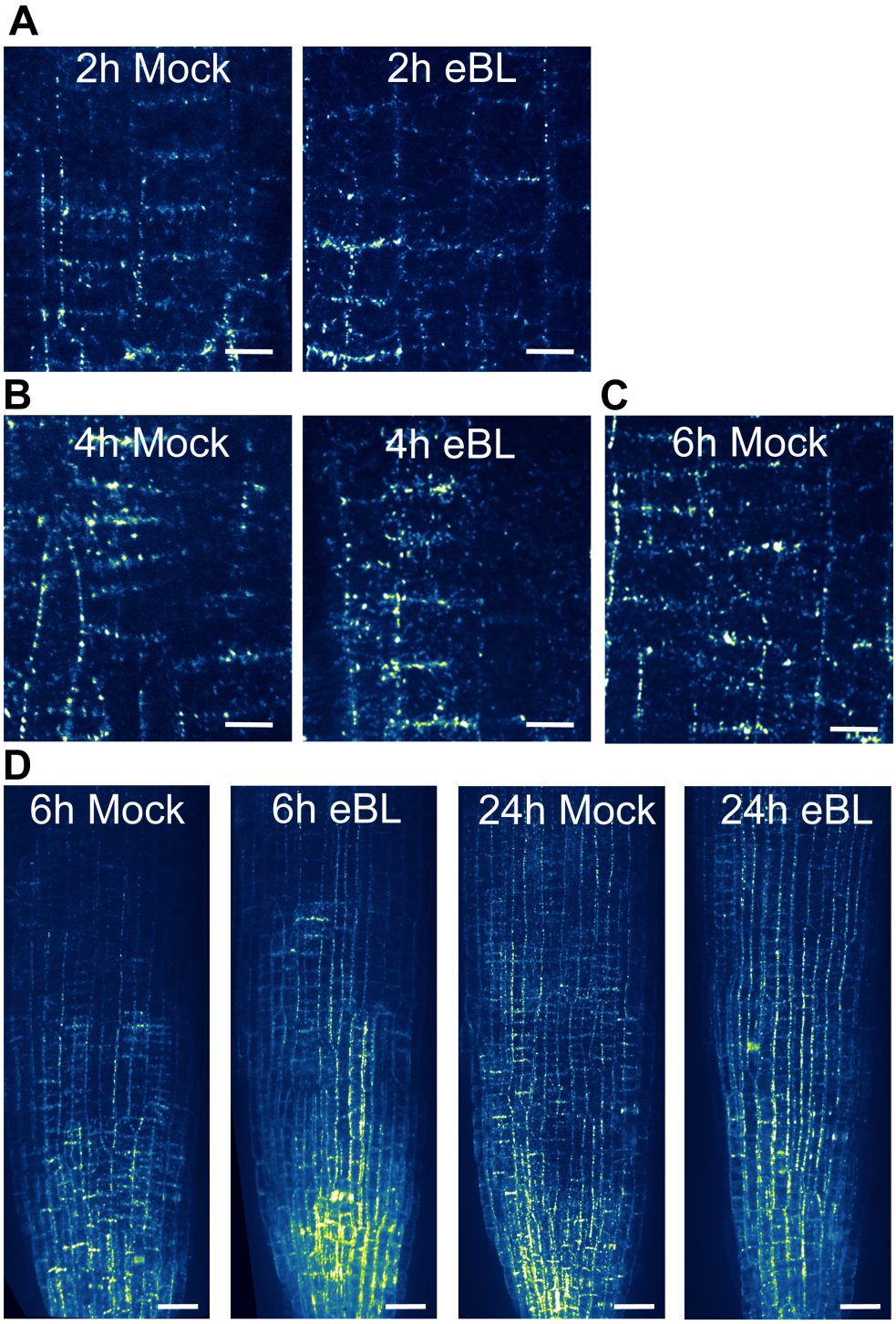
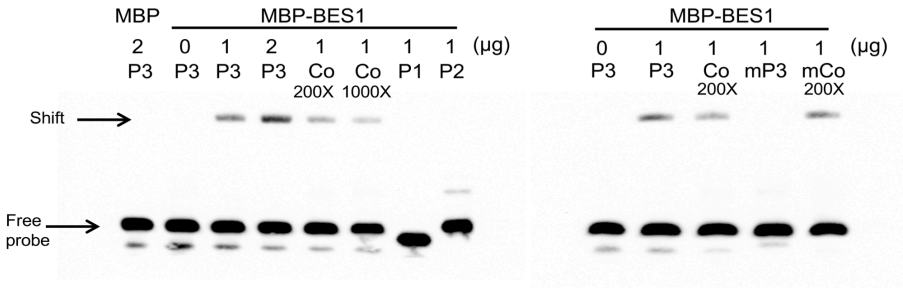


Figure S1. eBL alters the distribution of CLASP at cell edges, Related to Figure 1E. 2, 4h eBL treatments, 6h mock treatment showing GFP-CLASP punctae on transverse and longitudinal cell edges of epidermal cells in the meristem. Scale bars = 10μm in A-C, 30 μm in D

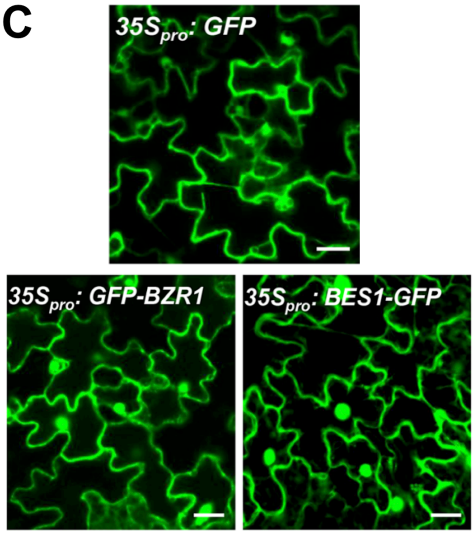
A

atttctttgcttccttctcttaatattttactaacagtaaaccttttaacacataaacaaaaatcactaatagtgcttttatttgttagacctataacaatatttataaagaattgtga
ggtgaccaatttcagaaagaaatataattatttaccatttgaccacaaatattgtttgactttaatttatcatatataggaagttcactcatatcatgttgaatatgctagtttggta
tgttattagagtctaggataaacataaactttaccctctgtcacctatcgattcaaatctaatctgttagtagagtcttaattcttttattttcataaatttgtctttttattgcctaat
ccaattgtaagtgactttgtaatcagcatcaaagtaaatgattgtattttgggaagtgataagcacatagaagtaaaagaagaaaaaaaggtgggaaagttttaagcatgat
tacttgtcaattacgctccgactctttggcccgcaccagctgtttaagatcgccggcgtgacaagtgacaacaattggccacgtgtgtgtgatttattatcttaagtttttgttattt
ctctgcgaattttaaagaaaatttgaacttgatcaaacttgtatgaattagaggggatgcaaaaataaatacacattatacaaacgagatggtatgaaggataacaaaaaa
aaaaaagatggtatgaagaccaaaatatattaagcatttgattaaactccgatcagctgacatattaacatgatcaaaggttaatgtttagacaaaaggcttttattagttgag
aatggtcttatctaattaaacaccacattcgtgtttataaataatcaaatctaatgaaccgacgtcgggtattatatcttatcagtggagtgatctaattaaactaatttcacaat
aactgattgattcgaatttagagttatttataacatatcttatttaagatagtcttttaaaattccaatgactagaaattaaatattcgtaatttggatttattccagctttgttagg
ttggtgatagacatagttaagattttctttttcttttttaatttaatgttatttaatgaaataaacattctcttgattttaatttaaagaaatttatttgtattgtttctctaaagaatttac
caaccagagtggaggcatttttttatcctatgttttgtccagatttggtgagatatttacttttttctttcggtttgatccaatttctaatcaaaatacgtatgagtagagcaacttttag
tttattttagtaaaattcactacatttttgttactatatgatgtggttcgactgcaccaatccgagttatccaaattagacactgatttttttttctcaaacatatgtaggtcgataaa
atcaaaaaatttatttgttttactccaaattaataccacattctttaacaaaaaaaaaagaaattttaaatttcaaattaatagctcctcaataaattaataattaagaattttatc
ttcatatatttcaaaagaacaattgctaaaactcctgtaattcactttattattttgttaataacccaaccggattaaaccaaccccctccgaatcggaaccggcggttaagttcag
agaccataacataatagtggtatgaaacagtaaatattaagaaaaacaagggcaagtaccaaagacgccagccaacccacatgctctcgtttatcttcttcaaaatgagtttag
tctctctactagtctctcgcctttactctttttcttttcttagtactaaaaattaaacgacacttctcccctcaaatccctttttctctctctaaacacatttacacacagaacacaacac
actctctctctctctctctctctctctacgattctcgctcactgttcatcgaatccgtcattggatcgatttggtgtgtgttttctttccaatacctctgattagagatttcagcaga
tctgaacggaggagcgtcagatcggaatcgtccgtcggtggtttggtaaaaaaaaaaATGGAGGAAGCTT

B



C



D

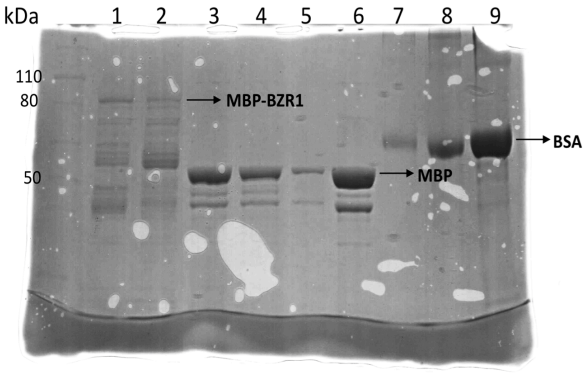


Figure S2. BES1 and BZR1 bind the *CLASP* promoter and downregulate *CLASP* expression. (A) Motifs and probes in the *CLASP* promoter, related to Figure 2A. Sequence analysis of the 2 kb *CLASP* promoter. The translational start codon (ATG) is highlighted with a red box. The BRRE motif (CGTGTG) and E-box (CANNTG) are underlined in red and black, respectively. P1, P2 and P3 are coloured in orange, red and blue. (B) BES1 binds the *CLASP* promoter, related to Figure 2B and C. EMSA for MBP-BES1 shows that BES1 binds to the P3 region (See Figure 2A) and that increasing

amounts of unlabelled P3 (Co 200X, Co 1000X) reduces binding, indicating specificity. Mutating the P3 BRRE motif (CGTGTG to AAAAAA) prevents this binding, as is evident by no band shift (mP3) and the failure of unlabeled mP3 (mCo) to compete with P3 for binding. (C) Transient expression of *$35S_{pro}$*: *GFP*, *$35S_{pro}$*: *GFP-BZR1* and *$35S_{pro}$*: *BES1-GFP* in tobacco leaves, related to Figure 2D. Scale bars = 10 μm. (D) Coomassie blue-stained gel of purified MBP and MBP-BZR1 proteins used in the EMSA, related to Figure 2B. Left of lane1: protein ladder. Lanes 1 and 2: two technical replicates of MBP-BZR1 big prep, 4 μl loaded per lane. MBP-BZR1 migrates at 87 kDa. Lanes 3 to 6: two replicates of MBP big prep, with 1 μl, 0.5 μl, 0.5 μl and 1 μl loaded per lane, respectively. Purified MBP protein is indicated at 50 kDa in lane 6. Lanes 7 to 9: BSA reference protein (1 μg, 5 μg and 20 μg respectively) for estimation of MBP and MBP-BZR1 protein concentrations. Purified MBP-BZR1 protein in lane 1 and MBP protein in lanes 3 and 4 were used in the subsequent EMSA experiment. The same purification scheme was used for MBP-BES1.

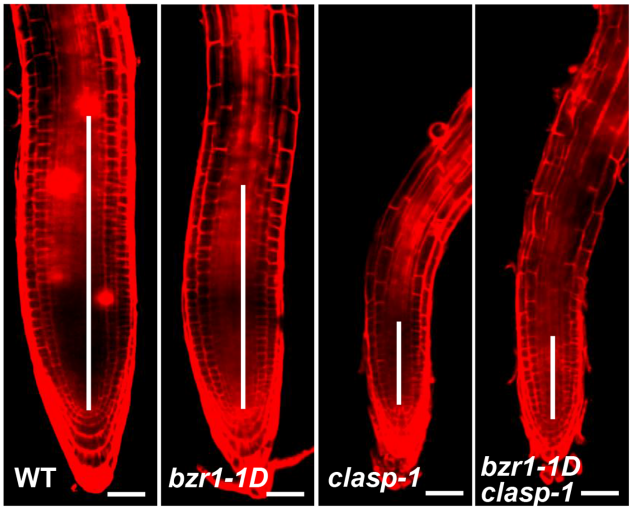


Figure S3. Meristem of *clasp-1 bzr1-1D* resembles that of *clasp-1*, Related to Figure 3D and Figure 3E. Root meristem morphology of wild type, *bzr1-1D*, *clasp-1* and *bzr1-1D clasp-1* stained with propidium iodide. Meristem regions are marked with white lines. Scale bars = 50 μm.

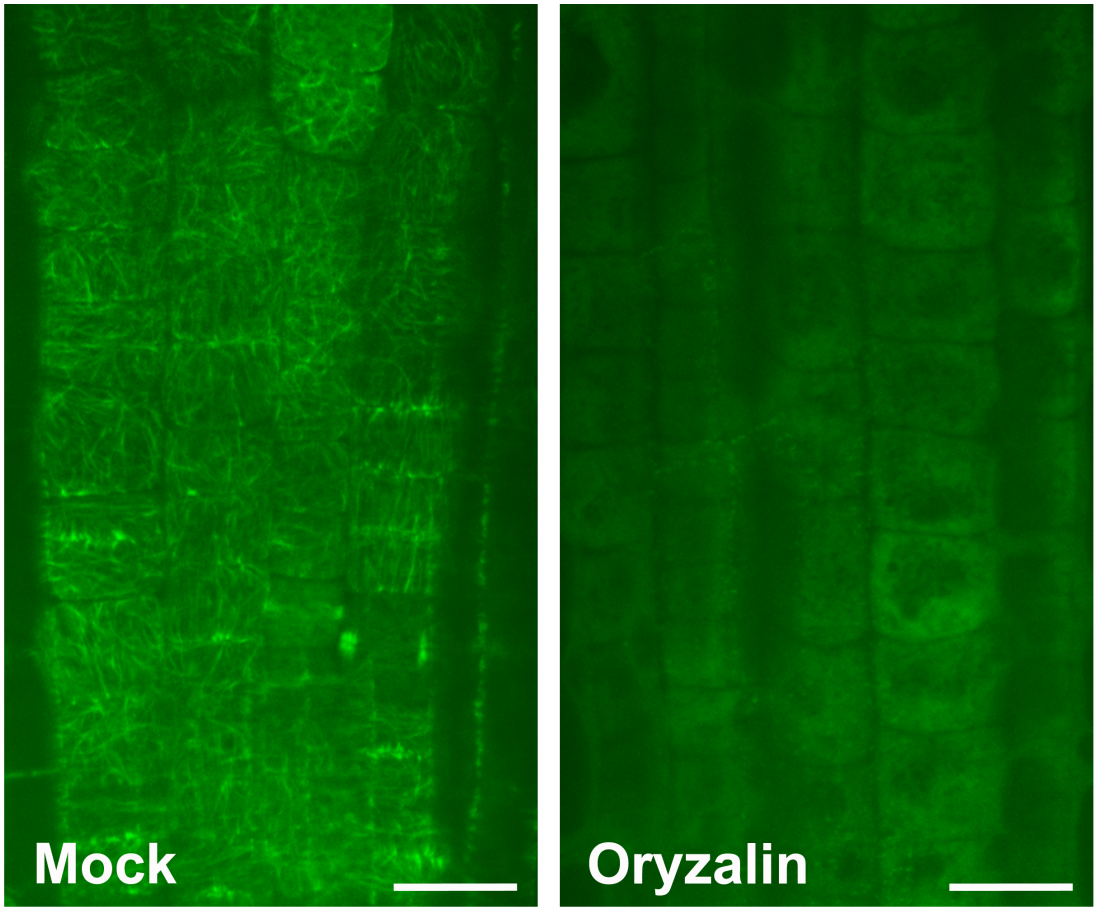


Figure S4. Oryzalin removes microtubules including CLASP-mediated bundles, Related to Figure 4H and Figure 4I. Compared to mock treatment, microtubules are absent after a 75-minute treatment with 40 μM oryzalin**.** Scale bars = 20 μm.

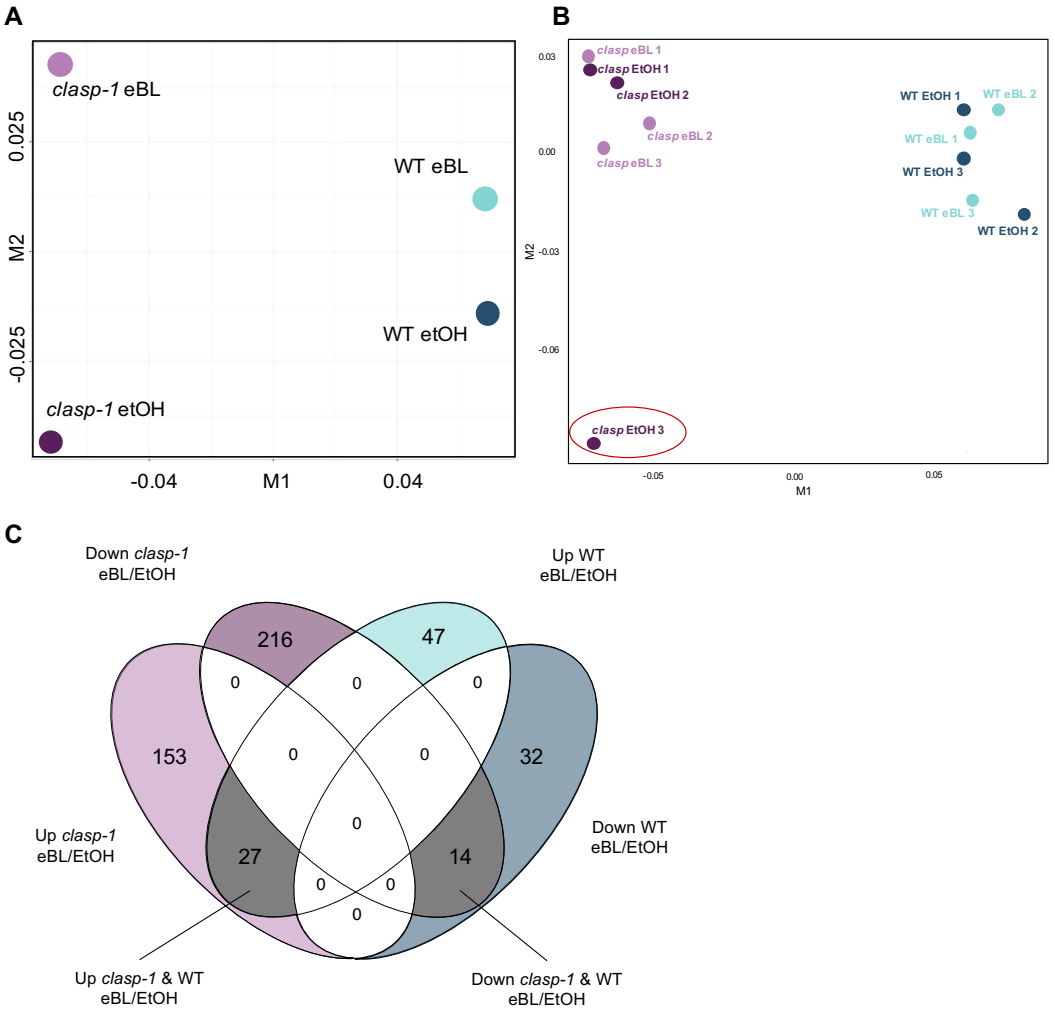


Figure S5. *clasp-1* mutants are transcriptionally distinct to wild type in response to eBL, Related to Figure 5. (A) Multidimensional scaling plot showing the relative similarity of the *clasp-1* and wild-type root tip transcriptomes in response to eBL. (B) Multidimensional scaling plot showing all bio-replicates of the *clasp-1* and wild-type (WT) root tip transcriptomes. One of the *clasp-1* ethanol control populations did not group with the rest (circled in red), and was not included in further analyses. (C) Venn diagram showing the number of genes in *clasp-1* and wild-type (WT) root tips showing higher or lower relative expression in eBL compared to mock (EtOH) treatments.

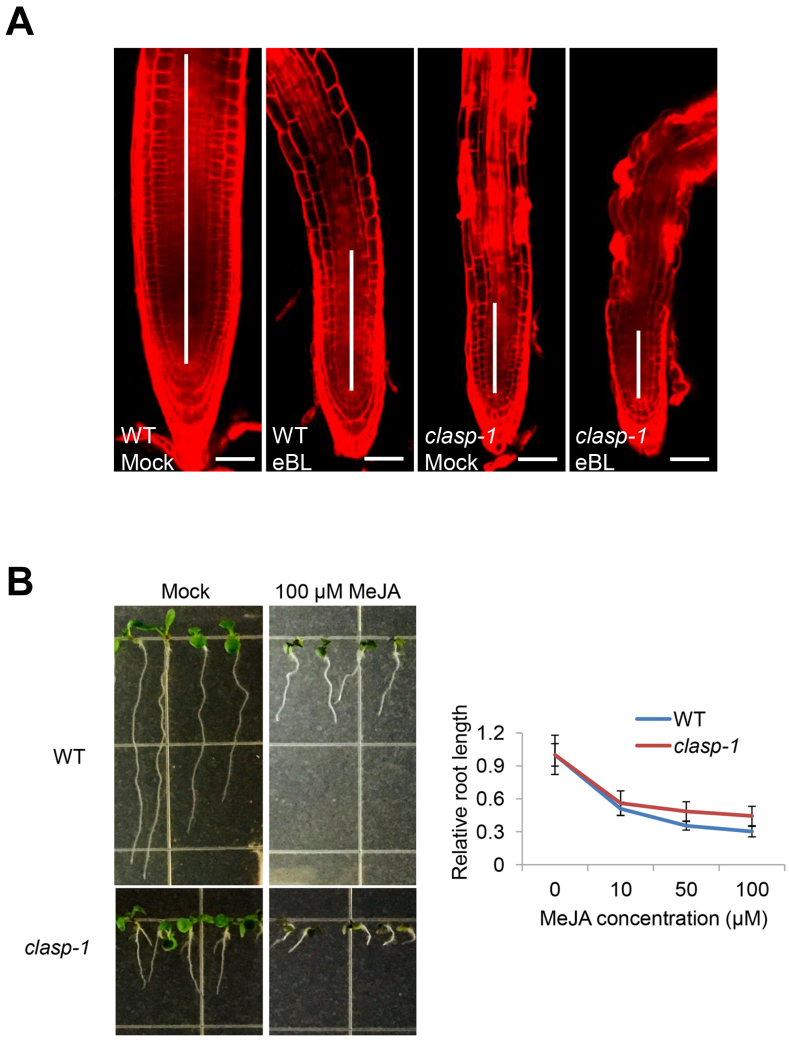


Figure S6. (A) Root meristem phenotypes of wild type and *clasp-1* mutants grown on medium with or without 10 nM eBL for 9 days, Related to Figure 6A and Figure 6B. Meristem regions are marked with white lines. Scale bars = 50 µm. (B) Wild-type and *clasp-1* response to methyl jasmonate (MeJA) treatment, related to Figure 6D. Seedling morphology and relative root lengths of wild type and *clasp-1* grown for 7 days on medium with or without 100 µM MeJA. Grid marks = 1 cm. (n=3, 30 seedlings for each genotype at each concentration). Error bars represent SD.

Category	Gene name	AGI	*clasp-1*/WT
Sterol biosynthesis	*FK* *	*AT3G52940*	0.69
	SMT2	*AT1G20330*	0.98
	DWF7 *	*AT3G02580*	1.26
	DWF5	*AT1G50430*	1.12
	DWF1	*AT3G19820*	0.96
BR-specific biosynthesis	*DET2*	*AT2G38050*	1.10
	DWF4 *	*AT3G50660*	2.98
	CPD	*AT5G05690*	1.11
	BR6ox1	*AT5G38970*	1.08
	ROT3 *	*AT4G36380*	2.63
BR inactivation	*BAS1* *	*AT2G26710*	2.09

Table S1. Fold changes in transcript accumulation of select genes involved in brassinosteroid homeostasis in *clasp-1* root tips relative to wild type, Related to Figures 5F, 6A and 6B. Transcripts that are significantly different in *clasp-1* and wild type (WT) are indicated with an asterisk (*). Significance is based on pairwise comparisons of transcript accumulation using Cuffdiff [S1].

Category	Gene name	AGI	*clasp-1*/WT
Polar auxin influx	*AUX1* *	*AT2G38120*	1.48
	LAX2 *	*AT2G21050*	0.76
	LAX3 *	*AT1G77690*	3.10
PIN Auxin transport	*PIN1* *	*AT1G73590*	1.21
	PIN2	*AT5G57090*	1.09
	PIN3 *	*AT1G70940*	1.33
	PIN7 *	*AT1G23080*	1.53
Cytokinin response and auxin inactivation	*ARR1* *	*AT3G16857*	1.60
	ARR12 *	*AT2G25180*	1.24
	SHY2 *	*AT1G04240*	3.02
	GH3.3	*AT2G23170*	-
	GH3.17 *	*AT1G28130*	1.41
	BRX	*AT1G31880*	-

Table S2. Fold changes in transcript accumulation of genes involved in modulating the auxin minimum at the root transition zone in *clasp-1* root tips relative to wild type, Related to Figure 6A and 6B. NA indicates that transcript accumulation was too low to quantify. Transcripts that are significantly different in *clasp-1* and wild type (WT) are indicated with an asterisk (*). Significance is based on pairwise comparisons of transcript accumulation using Cuffdiff [S1].

Name	Sequence	Notes
CLASP promoter (*CLASP*$_{pro}$) Forward	CCCAAGCTTCACATAAACAAAAATCACTAATAG	For the *CLASP*$_{pro}$:*GFP* and *CLASP*$_{pro}$:*GUS* reporters, with HindIII, pair with *CLASP* promoter (*CLASP*$_{pro}$) Reverse
CLASP promoter (*CLASP*$_{pro}$) Reverse	ACGCGTCGACTTTTTACCAAACCACCGAC	For the *CLASP*$_{pro}$:*GFP* and *CLASP*$_{pro}$:*GUS* reporters, with SalI, pair with *CLASP* promoter (*CLASP*$_{pro}$) Forward
smrsGFP Forward	ACGCGTCGACATGAGTAAAGGAGAAGAAC	For the *CLASP*$_{pro}$:*GFP* and *UBQ1*$_{pro}$:*GFP-MBD* reporters, with SalI, pair with smrsGFP Reverse
smrsGFP Reverse	CGGACTAGTTGCTGCTGCTGCTGCTGC	For the *CLASP*$_{pro}$:*GFP* and *UBQ1*$_{pro}$:*GFP-MBD* reporters, with SpeI, pair with smrsGFP Forward
MAP4-MBD Forward	CGGACTAGTATGTCCCGGCAAGAAGAAG	For the *UBQ1*$_{pro}$:*GFP-MBD* reporter, with SpeI, pair with MAP4-MBD Reverse
MAP4-MBD Reverse	CAAGAGCTCAGATCCCGGGCCCACCTCC	For the *UBQ1*$_{pro}$:*GFP-MBD* reporter, with SacI, pair with MAP4-MBD Forward
DWF4 Forward	CACGAGCAACGATATTGAAGTTC	qPCR, pair with *DWF4* Reverse, 314bp, used in [S2]
DWF4 Reverse	CCTAAGCTCTTCAACGGCTTTAG	qPCR, pair with *DWF4* Forward, 314bp, used in [S2]
CLASP Forward	ATTTCTGAAATGCTAAAGAG	qPCR, pair with *CLASP* Reverse, 186 bp
CLASP Reverse	CAATAATGGGACAATAACGC	qPCR, pair with *CLASP* Forward, 186 bp
MUSE3 Forward	GTGGGAGCAGAGACCAACTC	qPCR, pair with MUSE 3 Reverse, 176 bp, used in [S3]
MUSE3 Reverse	TGGCAACCCTCTCAACCATC	qPCR, pair with MUSE3 Forward, 176 bp, used in [S3]
BRI1 Forward	TTCCTCGGAGATTGCTCTGC	qPCR, pair with BRI1 Reverse, 197 bp
BRI1 Reverse	CCGGTGAATTTGTTCTCGGC	qPCR, pair with BRI1 Forward, 197 bp
CLASP promoter EMSA probe P1	TGAAGAAGATAAACGAGAGCATGTGGTTGGCTGGCGTC	For EMSA assay
CLASP promoter EMSA probe P1	GACGCCAGCCAACCACATGCTCTCGTTTATCTTCTTCA	For EMSA assay
CLASP promoter EMSA probe P2	CAAAATATATTAAGCATTTGATTAAACTCCGATCAGCTGACATATTAACATGA	For EMSA assay
CLASP promoter EMSA probe P2	TCATGTTAATATGTCAGCTGATCGGAGTTTAATCAAATGCTTAATATATTTTG	For EMSA assay
CLASP promoter EMSA probe P3	CGCCGGCGTGACAAGTGACAACAATTGGCCACGTGTGTGATTTATTAT	For EMSA assay, underlined is the BZR1 binding motif
CLASP promoter EMSA probe P3	ATAATAAATCACACACGTGGCCAATTGTTGTCACTTGTCACGCCGGCG	For EMSA assay, underlined is the BZR1 binding motif
mutated *CLASP* promoter probe P3	CGCCGGCGTGACAAGTGACAACAATTGGCCAaaaaaaTGTGATTTATTAT	For EMSA assay, underline shows the BZR1 binding motif mutated

mutated *CLASP* promoter probe P3	ATAATAAATCACAttttttTG GCCAATTGTTGTCACTTG TCACGCCGGCG	For EMSA assay, underline shows the BZR1 binding motif mutated
mutated *CLASP* promoter *(CLASP$_{mpro}$)* Forward	CAAGTGACAACAATTGG CCAaaaaaaTGTGATTTAT TATCTTAAG	For the *CLASP$_{mpro}$:GUS* reporter, paired with mutated *CLASP* promoter (*CLASP$_{mpro}$*) Reverse
mutated *CLASP* promoter *(CLASP$_{mpro}$)* Reverse	CTTAAGATAATAAATCAC AttttttGGCCAATTGTTGT CAC TTG	For the *CLASP$_{mpro}$:GUS* reporter, paired with mutated *CLASP* promoter (*CLASP$_{mpro}$*) Forward
BZR1-attB1	GGGGACAAGTTTGTACA AAAAAGCAGGCTTCATG ACTTCGGATGGAGCTA	For tobacco transient assay, gateway cloning into pDONR221 then pGWB5/6 for *35S$_{pro}$:BZR1-GFP* and *35Spro:GFP-BZR1*
BZR1-attB2	GGGGACCACTTTGTACA AGAAAGCTGGGTCACCA CGAGCCTTCCCAT	For tobacco transient assay, gateway cloning into pDONR221 then pGWB5 for *35S$_{pro}$:BZR1-GFP*, paired with BZR1-attB1
BZR1-attB2 (with stop codon)	GGGGACCACTTTGTACA AGAAAGCTGGGTTCAAC CACGAGCCTTCCC	For tobacco transient assay, gateway cloning into pDONR221 then pGWB6 for *35S$_{pro}$:GFP-BZR1*, paired with BZR1-attB1
BES1-attB1	GGGGACAAGTTTGTACA AAAAAGCAGGCTTCATG ACGTCTGACGGAGCA	For tobacco transient assay, gateway cloning into pDONR221 then pGWB5/6 for *35S$_{pro}$:BES1-GFP* and *35S$_{pro}$:GFP-BES1*
BES1-attB2	GGGGACCACTTTGTACA AGAAAGCTGGGTCACTA TGAGCTTTACCATTTCCA	For tobacco transient assay, gateway cloning into pDONR221 then pGWB5 for *35S$_{pro}$:BES1-GFP*, paired with BES1-attB1
BES1-attB2 (with stop codon)	GGGGACCACTTTGTACA AGAAAGCTGGGTTCAAC TATGAGCTTTACCATTTC	For tobacco transient assay, gateway cloning into pDONR221 then pGWB6 for *35S$_{pro}$:GFP-BES1,* paired with BES1-attB1

Table S3. Oligonucleotides used for cloning, quantitative real time PCR (qPCR), and Electrophoretic Mobility Shift Assays (EMSAs), Related to STAR Methods.

Supplemental References

S1. Trapnell, C., Roberts, A., Goff, L., Pertea, G., Kim, D., Kelley, D.R., Pimentel, H., Salzberg, S.L., Rinn, J.L., and Pachter, L. (2012). Differential gene and transcript expression analysis of RNA-seq experiments with TopHat and Cufflinks. Nat Protoc *7*, 562-578.

S2. Yan, Z., Zhao, J., Peng, P., Chihara, R.K., and Li, J. (2009). BIN2 functions redundantly with other Arabidopsis GSK3-like kinases to regulate brassinosteroid signaling. Plant Physiol *150*, 710-721.

S3. Hacham, Y., Holland, N., Butterfield, C., Ubeda-Tomas, S., Bennett, M.J., Chory, J., and Savaldi-Goldstein, S. (2011). Brassinosteroid perception in the epidermis controls root meristem size. Development *138*, 839-848.